CLAIMS

1. A method for the production of a mature recombinant protein into the culture medium of an eukaryotic cell line genetically transfected with a cloned precursor cDNA sequence, comprising an incubation of said cell line in the cell culture medium wherein alkanoic acids, their derivatives or salts thereof have been added for a time of at least 24 hours.

2. A method according to claim 1 wherein said cDNA sequence encodes for a protein precursor.

3. A method according to claim 2 wherein said precursor cDNA sequence encodes for the human Pre-prourokinase.

4. A method according to claim 1 wherein said mature recombinant protein is two chain-*uPA* (*tc-uPA*).

5. A method according to claim 4 wherein the two chain *uPA* is *HMW*.

6. A method according to claim 4 wherein the two chain *uPA* is *LMW*.

7. A method according to claim 4 wherein said alkanoic acids and/or their salts and/or derivatives thereof are chosen among: butyric acid, sodium butyrate, sodium propionate, magnesium butyrate, tributyrin and phenyl-butyrate.

8. A method according to claim 7 wherein said eukaryotic cell line is a mammalian cell line chosen among: HEK-293, CV-1, COS, BSC-1, MDCK, A-431, CHO, BHK, CHO-Messi.

9. A method according to claim 8 wherein said time is comprised between 48 and 200 hours.

10. A method according to claim 8 wherein said cell culture is serum-free.

11. A method according to claim 8 wherein said incubation is performed at a temperature equal or lower than 37°C.

12. A process for the production of recombinant *tc-uPA* comprising the following steps:

a) culturing genetically manipulated CHO cells stably transfected with the Pre-prourokinase cDNA in a culture media comprising alkanoic acids or their derivatives or salts thereof, at a temperature comprised between 30°C and 37°C;

b) continuing said cell-culture for a period of time of at least 24 hours;

c) recovering the cell culture supernatant.

13. A process according to claim 12 wherein said period of time in step b) is comprised between 72 and 150 hours.

14. A process according to claim 12 wherein cell viability of said CHO cell-culture in step b) is at least 70%.

15. A process according to claim 12 wherein said temperature is comprised between 33°C and 35°C.

16. A process according to claim 12 wherein said alkanoic acid derivative is chosen among: butyric acid, sodium butyrate, sodium propionate, magnesium butyrate, tributyrin, phenyl butyrate, at concentration comprised between 0.1 mM and 20 mM.

17. A process according to claim 16 wherein said CHO cells are CHO-Messi cells.

18. A process according to claim 17 wherein in step a) said culture media is a serum free culture medium.

19. A process for the isolation of recombinant *HMW* and/or *LMW tc-uPA* from an exhausted culture media of genetically engineered CHO cells characterized by using the cell culture supernatant obtained according to claim 17.

20. A process according to claim 19 wherein said isolation comprises a ion-exchange chromatography.

21. A process according to claim 20 for the separation of recombinant *HMW* from *LMW tc-uPA* further comprising the steps of:

d) acidification of the cell culture supernatatant with a weak acid to pH values comprised between 5 and 5.8, optionally adding a non-ionic detergent;

e) contacting the acidified supernatant with a ion-exchange chromatography column at pH values comprised between 5.5 and 6.5;

f) releasing the *LMW tc-uPA* by addition of a buffer solution with a pH value comprised between 5.5 and 6.5, comprising a monovalent ion in concentration comprised between 200 and 300 mM;

g) releasing the *HMW tc-uPA* by addition of a buffer solution with a pH value comprised between 6-7.5, comprising monovalent ions in concentration of at least 400 mM.

22. A process according to claim 21 wherein the acidified supernatant in step d) is additionally filtered.

23. A process according to claim 21 wherein said isolation further comprises a benzamidine chromatography.

24. A process according to claim 23 for the purification of recombinant *tc-uPA HMW* comprising the steps of:

g') contacting the released *HMW tc-uPA* containing buffer solution in step g) with a benzamidine column, at pH values comprised between 6.2 and 6.8

g") releasing the *tc-uPA HMW* with a buffer solution with a pH value comprised between 3.8 and 4.2, further comprising monovalent ions in concentration comprised between 300 and 500 mM;

g"') further optionally contacting the released *tc-uPA HMW* with a gel-filtration column and releasing of the *HMW tc-uPA* with a low-salt solution buffer at pH values comprised between 4 and 7.

25. A process according to claim 23 for the purification of recombinant *tc-uPA LMW* further comprising the additional steps of:

f') contacting the released *LMW tc-uPA* containing solution obtained in step f), with a benzamidine column, at pH values comprised between 6 and 8;

f") releasing the *tc-uPA LMW* with a buffer solution with pH values comprised between 3.8 and 4.2 further comprising monovalent ions in concentration comprised between 300 mM and 500 mM;

f"') further optionally contacting the released *tc-uPA LMW* with a gel-filtration column and releasing the *LMW tc-uPA* with a low-salt solution buffer at a pH comprised between 4 and 7.

26. Recombinant *tc-uPA* obtainable by the process according to claim 12.

27. Recombinant *tc-uPA* obtainable by the process according to claim 18.

28. Recombinant *HMW* and *LMW tc-uPA* product obtainable by the process according to claim 21.

29. Recombinant *HMW* and *LMW tc-uPA* product obtainable by the process according to claim 23.

30. Recombinant purified *HMW tc-uPA* obtainable by the process according to claim 24.

31. Recombinant purified *LMW tc-uPA* obtainable by the process according to claim 25.

32. A method for the treatment of thromboembolytic disorders wherein recombinant *HMW tc-uPA* according to claim 30 is used.

33. A method for the treatment of thromboembolytic disorders wherein recombinant *LMW tc-uPA* according to claim 31 is used.

34. A method according to claim 32 wherein said disorders are chosen among: peripheral arterial occlusion (PAOD), catheter clearance, pulmonary embolism, deep venous thrombosis.

35. A method according to claim 33 wherein said disorders are chosen among: peripheral arterial occlusion (PAOD), catheter clearance, pulmonary embolism, deep venous thrombosis.

36. A method for the treatment of myocardial infarction wherein *HMW tc-uPA* according to claim 30 is used.

37. A method for the treatment of myocardial infarction wherein *LMW tc-uPA* according to claim 31 is used.

38. Pharmaceutical compositions comprising as an active agent the recombinant *HMW* tc-uPA according to claim 30.

39. Pharmaceutical compositions comprising as an active agent the recombinant *LMW* tc-uPA according to claim 31.